PHOTOPERIODISM AND THE ANNUAL TESTICULAR CYCLE OF THE BOBOLINK (DOLICHONYX ORYZIVORUS), A TRANSEQUATORIAL MIGRANT, AS COMPARED WITH TWO TEMPERATE ZONE MIGRANTS[1]

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It is now quite generally accepted that seasonal changes in day-length play an important role in the temporal regulation of several annual cyclic phenomena in many birds of the northern hemisphere. Best known is the effect of photoperiods on the annual cycle of the testes, but evidence is accumulating that there is an effect also on a metabolic cycle apparently peculiar to migratory species, an effect perhaps on migration and perhaps also on the molt cycle (summarized in Farner, 1959).

Transequatorial migrants are of special interest in generalizing from known photoperiodic effects because they are exposed annually to two day-length cycles rather than one (*cf.* Fig. 1), and yet, like all other birds which breed above tropical or subtropical latitudes, they have only one reproductive period each year. Unfortunately, very little experimental work has so far been done on transequatorial migrants. Marshall and Serventy (1959) showed that photoperiods apparently do not influence either the gonadal or molt cycles of a shearwater which, following a breeding season off southeastern Australia, migrates across the equator to the North Pacific Ocean, as far as the Aleutian Islands. Preliminary studies (Engels, 1959) indicated a photoperiodic effect on the testicular cycle of the bobolink (*Dolichonyx oryzivorus*), which breeds in North America above Lat. 40° N. and after a transequatorial migration to South America occupies an area between approximately Lat. 10° and 30° S. These preliminary experiments were in part deficient in design, and certain conclusions drawn from them must be modified in view of the further experimental results now to be reported.

MATERIALS AND METHODS

Sixteen bobolinks (*Dolichonyx oryzivorus*), 6 slate-colored juncos (*Junco hyemalis*), and 6 white-throated sparrows (*Zonotrichia albicollis*) were used in the experiments. The juncos and white-throated sparrows, representative of Temperate Zone migrants, were captured in February and March from populations wintering in the vicinity of Chapel Hill, North Carolina, and were held through the following summer in an outdoor aviary. Seven of the bobolinks were caught in May on their northward migration, one near Raleigh, N. C., the others near Chapel Hill. Like the juncos and white-throated sparrows, these were held in an outdoor aviary through the summer and thus were exposed to nearly normal

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[1] Aided by grants from the National Science Foundation (G-6163) and the Faculty Research Council of the University of North Carolina.

day-lengths until the beginning of experimental lighting on October 1. Nine of the bobolinks were captured in early September near Wilmington, N. C., while on their southward migration, just a few weeks before the start of experimental lighting. Five of the 7 birds taken in May were returned to the aviary in the following May, after 7 to 8 months of artificial day-lengths during which they passed through a complete testicular cycle. They spent the summer again in the outdoor aviary and were used in further experiments beginning in the next autumn—in the tables and discussion these birds are referred to as "second-year experimentals." (Since 5 of the 16 bobolinks were each used twice, there are 21 entries in Table II.)

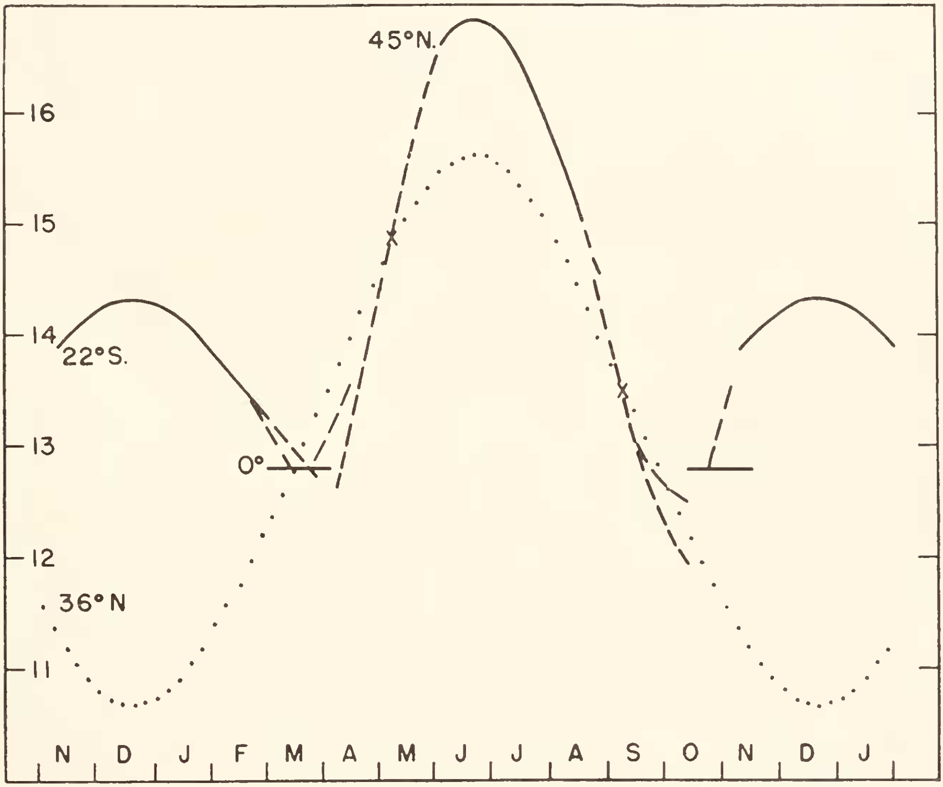

FIGURE 1. Annual cycles of day-lengths (hours between beginning of morning civil twilight and end of evening civil twilight) experienced by bobolinks: *solid lines*, near the center of their "wintering" grounds, at Lat. 22° S.; near the center of their breeding grounds, at Lat. 45° N.; and at the equator, Lat. 0°, during their transequatorial passages; *broken lines*, during migrations (approximate); *x*, time of capture of spring and fall migrants (early May and early September) in North Carolina; *dotted line*, the cycle (at Lat. 36° N.) experienced by captive bobolinks in aviary at Chapel Hill, N. C.—the general form of this cycle is that experienced by all Temperate Zone migrants as well as by non-migrant residents of middle latitudes. Curves for hours between sunrise and sunset would be lower by about one hour, varying from about ¾ hour at the equator and at Lat. 22° S. in December to about 1¼ hours at Lat. 45° N. in June. (From data in: Tables of Sunrise, Sunset and Twilight: Supplement to the American Ephemeris, 1946; U. S. Naval Observatory. Government Printing Office, Washington, D. C.)

TABLE I

Responses of juncos and white-throated sparrows to 14-hour photoperiods following 8 weeks (October 1–November 26) of shorter periods; photoperiods from March to October 1 were those natural at Lat. 36° N.

Species	Photoperiod Oct. 1–Nov. 26	Date killed	Vol. of testes	Spermatogenesis
Junco	10-hour	Jan. 16	295 mm.^{3}	sperm in bundles
Junco	10-hour	Jan. 16	247	sperm in bundles
Junco	10-hour	Jan. 16	230	sperm in bundles
Junco	12-hour	Jan. 16	1.2	inactive
Junco	12-hour	Feb. 6	2.1	inactive
Junco	12-hour	March 6	1.2	inactive
White-throated sparrow	10-hour	Jan. 16	440	sperm in bundles
White-throated sparrow	10-hour	Jan. 16	403	sperm in bundles
White-throated sparrow	10-hour	Jan. 16	226	sperm in bundles
White-throated sparrow	12-hour	Jan. 16	2.4	inactive
White-throated sparrow	12-hour	Feb. 6	8.2	spermatocytes
White-throated sparrow	12-hour	March 6	3.1	inactive

Different groups of birds, two to four in each group, were exposed to experimental photoperiods beginning October 1, as follows: 10 hours daily (10 hours light, 14 hours dark) continuously for 7 to 8 months; 14 hours daily, also continuously; 10 hours, 11 hours, or 12 hours daily for eight weeks, followed by 14 hours daily until May (about 6 months); and, in an outdoor aviary, changing day-lengths normal to Lat. 36° N.—this latter regime may properly be termed an experimental treatment for transequatorial migrants since it differs so radically from that which they would have encountered in the free state (*cf.* Fig. 1).

The conditions of confinement during the experimental treatment for the juncos, white-throated sparrows and those bobolinks caught during May were similar to those used in the preliminary experiments (Engels, 1959); that is, two to four birds together in a cage measuring 24 × 24 × 36 inches. The other bobolinks, including the second-year experimentals, were confined individually in Hendryx "finch breeding cages" measuring 8 × 9 × 16 inches. Terramycin was added to the drinking water supplied to these birds. Food, during most of the experiments, consisted of a chick laying mash (Purina Layena); during the last few months we changed to a mash prepared for "game" birds (also Purina).

The development of black pigment in the beak was taken as the criterion of testicular recrudescence in the bobolinks. The first appearance of this pigmentation probably coincides with that stage of the spermatogenic cycle when most of the spermatocytes are undergoing the first meiotic division. However, it should be noted that the observations have to do with evidence of the cycle of production of male sex hormone, which apparently directly causes beak pigmentation (Engels, 1959), and only by inference with the spermatogenic cycle.

EXPERIMENTAL RESULTS

The results of the experiments as given in Tables I and II may be summarized as follows:

Juncos and white-throated sparrows

Valid comparison may be made between these Temperate Zone migrants (Table I) and the bobolinks caught in May and subjected to similar artificial photoperiods (Table II, spring captures, Groups B and D). Exposed to 14-hour photoperiods after 8 weeks of 10-hour photoperiods, juncos and white-throated sparrows had already completed spermatogenesis by January 16, whereas bobolinks first developed beak pigmentation between mid-February and early March, 4 to 7 weeks later. On the same 14-hour photoperiod following 8 weeks of 12-hour photoperiods, testes of juncos and most white-throated sparrows remained in the winter inactive state at least to early March, by which time bobolinks had already developed beak pigmentation.

TABLE II

Response of male bobolinks to various photoperiods; beak pigmentation indicates production of male sex hormone and, inferentially, progress of spermatogenesis at least to first (meiotic) division of spermatocytes. ("Spring captures" and "2nd-year experimentals" experienced the natural day-lengths of Lat. 36° N. for at least four months before start of experiments on October 1)

Group	Category	Photoperiods		Beginning of beak pigment*
		Oct. 1–Nov. 26	Dec.–May	
A	autumn cap.	10-hour	10-hour	May 16
	2nd-yr. exp.	10-hour	10-hour	May 11
B	spring cap.	10-hour	14-hour	Feb. 13
	spring cap.	10-hour	14-hour	Feb. 20
	autumn cap.	10-hour	14-hour	March 12
	autumn cap.	10-hour	14-hour	March 16
	2nd-yr. exp.	10-hour	14-hour	Feb. 17
C	spring cap.	11-hour	14-hour	Feb. 27
	spring cap.	11-hour	14-hour	Feb. 27
D	spring cap.	12-hour	14-hour	Feb. 20
	spring cap.	12-hour	14-hour	March 6
	autumn cap.	12-hour	14-hour	March 2
	autumn cap.	12-hour	14-hour	March 16
	2nd-yr. exp.	12-hour	14-hour	Feb. 17
E	autumn cap.	14-hour	14-hour	—**
	autumn cap.	14-hour	14-hour	—**
	2nd-yr. exp.	14-hour	14-hour	Feb. 17
F	autumn cap.	Natural to Lat. 36° N.		April 20
	autumn cap.	Natural to Lat. 36° N.		April 20
	spring cap.	Natural to Lat. 36° N.		April 1
	2nd-yr. exp.	Natural to Lat. 36° N.		April 1

* Dates indicate end of interval (4 to 7 days) during which beak pigmentation appeared.
** Beak still neutral, May 25.

Bobolinks

Lumping together the three categories in Table II (spring captures, autumn captures, second-year experimentals) for the various groups, we may note the following significant points. When exposed to constant 10-hour photoperiods (Group A), beak pigmentation did not appear until mid-May. When, after 8 weeks on 10-hour photoperiods, the length of the photoperiod was increased to 14 hours, beak pigmentation developed in the various individuals between mid-February and mid-March (Group B), two to three months earlier than in the first group. Similarly, when the 14-hour photoperiod was preceded by 8 weeks of either 11-hour or 12-hour photoperiods, beak pigmentation appeared variously from mid-February to mid-March (Groups C, D). However, on continuous exposure to 14-hour photoperiods from October 1 (Group E), without prolonged previous exposure to short days, only one of three birds (a second-year experimental) developed pigment in the beak; two birds caught a few weeks before treatment began still lacked beak pigmentation in late May, when the experiment was terminated. Bobolinks held in the outdoor aviary (Group F) developed beak pigmentation in April, definitely later than most of the birds on artificial photoperiods, but from 4 to 7 weeks earlier than those continuously on 10-hour photoperiods. Song was heard from the aviary beginning in the latter part of March, when several inches of snow lay on the ground.

In general, beak pigmentation began somewhat later in birds caught in the autumn than in the other categories.

Discussion

Some preliminary experiments on bobolinks (Engels, 1959) seemed to show that the rate of response to 14-hour photoperiods depended inversely on the length of the photoperiod to which males had been exposed previously during the postnuptial, photorefractory phase of their cycle. That is, on 14-hour photoperiods beak pigmentation developed earlier in males which had been previously exposed to 10-hour photoperiods, several weeks later in males previously on 12-hour photoperiods. The difference is not apparent in the present experiments. However, birds used in the earlier study had been held on continuously long photoperiods (16-hour) throughout the summer, rather than on natural day-lengths as in the present study, and it is suggested that this difference in preliminary treatment is responsible for the difference in results. The idea is held, tentatively, that the reinforcement of photorefractoriness induced by some months of previously long days was overcome sooner on the shorter (10-hour) photoperiod. Further study is necessary to determine if we have to do here with any facet of the photoperiodic mechanism as operative in nature.

The relatively rapid response of juncos and white-throated sparrows to long photoperiods in early winter following a few weeks of short photoperiods in autumn (Table I) is characteristic of Temperate Zone migrants. Bobolinks treated in identical manner, however, were quite slow to show a response (Table II, B–D). It would appear that the cycle in these transequatorial migrants strongly resists the accelerating influence of long days, and that some process in the mechanism of response even on long days requires a rather long time, several weeks longer than in Temperate Zone migrants. Farner (1954) expected such a retardation of testicular recrudescence in response to long days on the part of transequatorial migrants, and he postulated for them (p. 29) "a characteristically long refractory period." Although we may get involved in semantic difficulties here, the present experiments suggest rather that the retardation could be due, at least to some considerable extent, to a very slow rate of response following the termination of refractoriness. In other words, the value of Farner and Wilson's (1957) constant *k* for testicular growth may be very much lower in bobolinks, especially on long photoperiods, than in Temperature Zone migrants.

Wolfson and Westerhoff (1960) in a short summary report on photoperiodic responses of *Dolichonyx* conclude, as had I previously (Engels, 1959), that a preparatory phase (= photorefractory phase) exists in this transequatorial migrant and that, moreover, this phase "may be regulated by the short days during the period of the autumnal equinox." They further conclude that "compared with temperate species, the duration of the period of short days required may be longer." This seems to me to be an unnecessary extrapolation and quite probably an inaccurate estimate of the operation of the mechanism in nature. In all Temperate Zone migrants so far investigated, the refractory period is terminated in nature by mid-November (Farner, 1954, p. 30; Farner, 1959, p. 732). Study of Figure 1 will show that it would be virtually impossible for migrating bobolinks to continue to experience beyond mid-November the short days of low or middle latitudes north of the equator. (See also final paragraph of this discussion.)

It may be of interest to note especially the responses of those bobolinks designated in Table II as second-year experimentals. These are the same birds which a year earlier were the "spring captures" in Groups B, C and D: as such, following several months of experimental lighting, they all had gone through a relatively sudden post-nuptial molt in May, with accompanying disappearance of the black pigmentation characteristic of the beak in nuptial dress. In nature these events would not have occurred until about two months later. After another summer, then, in the outdoor aviary, they were again exposed to artificial photoperiods as "second-year experimentals." The three which had been distributed one each in Groups B, D and E developed beak pigmentation almost simultaneously during the 7-day interval ending February 17. I would not emphasize the relatively early date nor the coincidence of response (both of which may have some significance), but rather the appearance of beak pigment (also full song) in the one second-year experimental of Group E. This was the only one of 7 male bobolinks (including four birds of the preliminary experiments—Groups A, G; pp. 761, 762, Engels, 1959) on continuously long photoperiods which developed beak pigmentation. We may at least suspect that the abnormally long interval since the last testicular regression, perhaps together with the relatively shorter days of late summer and early fall (see also last paragraph, below), permitted this bird to pass through the refractory phase and eventually to respond to the stimulus of the 14-hour photoperiod.

Despite the obvious difficulty of interpretation of these second-year experimentals, it seems to me that the general picture revealed in these experiments is one which fits the requirements of a photoperiodic mechanism as the timer for annual recrudescence of the testes in a transequatorial migrant: (1) the initiation of those processes leading to release from photorefractoriness, during the relatively short days of early northern autumn and during the southward passage through the equatorial region; (2) following eventual release from photorefractoriness, regrowth of the testes at a slow rate during the southern hemisphere summer when day-lengths, though declining, are still relatively long; correlated (3) with processes, the regulation of which is largely unknown, leading to migration. Migration itself exposes the birds, once past the equator, to photoperiods of rapidly increasing length (*cf.* Fig. 1).

We may note here that bobolinks held captive in the northern hemisphere (Lat. 36° N.) and exposed to natural day-lengths (and temperatures) showed a revival of the testicular cycle by April (Table II, F). The first flocks of bobolinks (all males) normally arrive from the south at this latitude about May 1, at about the time at which our locally wintering juncos and white-throated sparrows begin their northward migration. Thus, the testicular cycles of these captive bobolinks were approximately in phase, and it may be assumed that if the birds had been released, they could have reached the breeding ground at about the proper time and in breeding condition. It is therefore suggested that, as far as the photoperiodic mechanism is concerned, bobolinks are not required to "winter" below the equator; they could establish a wintering population in the northern hemisphere. The reverse would not seem to be true of such Temperate Zone migrants as juncos and white-throated sparrows, without readjustment of their photoperiodic mechanisms. Both by reason of their inability to overcome refractoriness on intermediate day-lengths (about 12 hours; *cf.* Table I) and their rapid response to long days when released from refractoriness, they probably could not extend their wintering range to and beyond the equator and still maintain that regularity of the annual cycle which leads to the coincidence of one annual reproductive period with a favorable climatic season.

Finally, it should be pointed out that bobolinks may usually not experience such a long period of such relatively short days as used in the present experiments, especially if any appreciable part of the morning and evening twilight periods adds to the effective length of the natural photoperiod (Fig. 1), as is almost certainly true. If so, they must be able to pass through the photorefractory phase and become photoreceptive on photoperiods even longer than 12 hours. It will be of interest to test this possibility.

SUMMARY

1. Bobolinks exposed to 10-hour photoperiods daily through the fall, winter and spring did not develop the beak pigmentation characteristic of the male nuptial dress until early in May. Others exposed at the same time to 14-hour photoperiods daily failed to develop beak pigmentation. But when exposure to 14-hour photoperiods was preceded by 8 weeks (October–November) of 10-, 11- or 12-hour photoperiods daily, pigmentation of the beak began variously between mid-February and mid-March.

2. Juncos and white-throated sparrows exposed to 14-hour daily photoperiods following 8 weeks (October–November) of 10-hour daily photoperiods had completed spermatogenesis by mid-January; but in others for which the preliminary photoperiod had been 12 hours the testes remained inactive.

3. Bobolinks held throughout the fall, winter and spring in an outdoor aviary, on changing day-lengths natural at Lat. 36° N., developed beak pigmentation in April, thus being approximately in phase, in their testicular cycles, with locally wintering juncos and white-throated sparrows.

4. The results are interpreted to mean that bobolinks, unlike typical Temperate Zone migrants, are able to overcome post-nuptial photorefractoriness on days of intermediate length, such as would be encountered during transequatorial migration, but that regrowth of the testes through the long days of the southern hemisphere summer proceeds at a rate much slower than that demonstrated by Temperate Zone migrants on similarly long days, and that full recrudescence is thus delayed, probably beyond the onset of northward migration.

5. It is further suggested that as far as the photoperiodic mechanism is concerned bobolinks could establish a wintering population in the northern hemisphere; but juncos and white-throated sparrows could not extend their wintering range into the southern hemisphere without readjustment of this mechanism.

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